Ruolo degli antiossidanti enzimatici e non enzimatici nello stress ossidativo

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Structure of Chromatin in Spermatozoa

- In the mature spermatozoon, DNA replication and transcription are terminated due to an exchange of DNA binding histones for, in human, two types of protamines (P1 and P2, respectively)
- Besides inactivating DNA functions, the incorporation of protamines allows a tighter packaging of the DNA fibers, rendering the mature sperm nucleus a volume estimated to be approximately one-sixth that of a somatic cell nucleus.
- The condensed state is believed to create an almost water-free, crystalline structure
- Once the sperm nucleus has been delivered into the ooplasm, the condensed nucleus must decondense rapidly to release the DNA for formation of a paternal pronucleus. Any abnormal change in the structural organization can cause delays or defects in the delivery of the paternal DNA
- Furthermore, any damage to the DNA during the transition from the testicle to the oocyte cannot be repaired until the DNA is accessible for DNA repair systems in the ooplasm. The risk of error during the repair process increases with the number of DNA strand breaks in an individual sperm nucleus.

(Lars Björndahl and Ulrik Kvist, E. Baldi and M. Muratori (eds.), 2014)

Function of sperm chromatin structural elements in fertilization and development

W.S. Ward, Mol Hum Reprod 2010

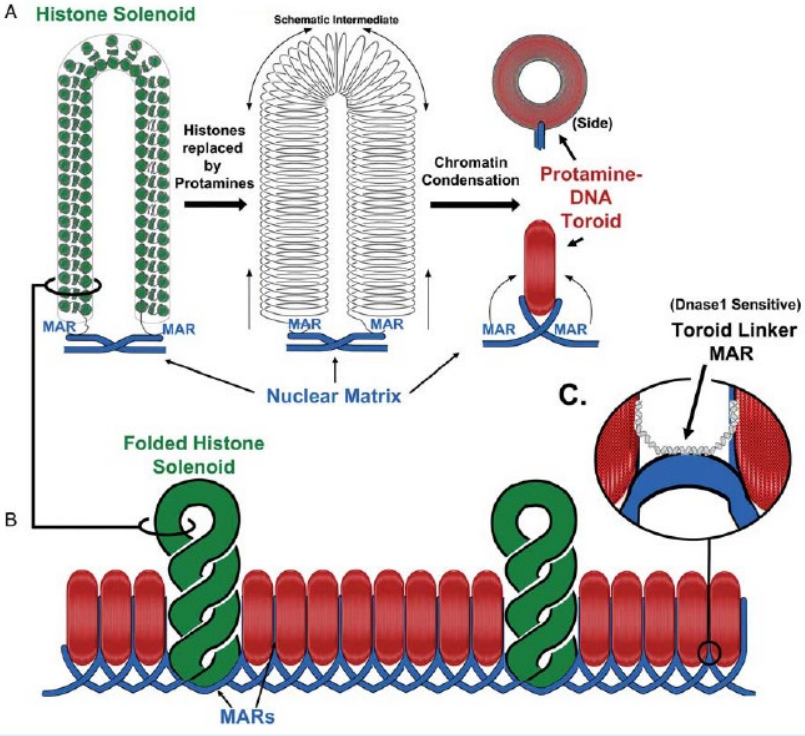


Figure 1 Three major structural elements of sperm chromatin.

(**A**) During spermiogenesis, histones are replaced by protamines, condensing the DNA into tightly packaged toroids. Each protamine toroid is a loop domain (part A of this figure is modified from a similar figure published in Ward, 1993). (**B**) Protamine toroids may be organized by stacking side to side. Recent evidence suggests that some large tracts of DNA retain histone (green solenoid). These may be entire loop domains that are not condensed by protamines. (**C**) The DNA strands that link the protamine toroids are nuclease sensitive, and may be bound to histones, as well. MAR, matrix attachment region

Alterations in the sperm structure

- Sperm DNA is primarily packaged tightly within the sperm head by protamines (Oliva and Dixon, 1991; Dadoune, 1995), which is conducive to a largely hypermethylated genome and a transcriptionally silent state.

- The localization and modification of retained histones (5–15% in humans) may reflect regions important for early embryonic development (Gatewood et al., 1987; Hammoud et al., 2009; Miller et al., 2010), including developmental genes, transcription factors, microRNAs (miRNAs) and imprinted genes.

- Abnormal sperm DNA methylation signatures have largely been associated with MF infertility, particularly noted in oligozoospermic patients and observed at imprinted and developmental genes located at retained histones (Hammoud et al., 2010, 2011).

M.M. Denomme et al, Hum Reprod, 2017

The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy

Parviz Gharagozloo [1,*] and R. John Aitken [2]

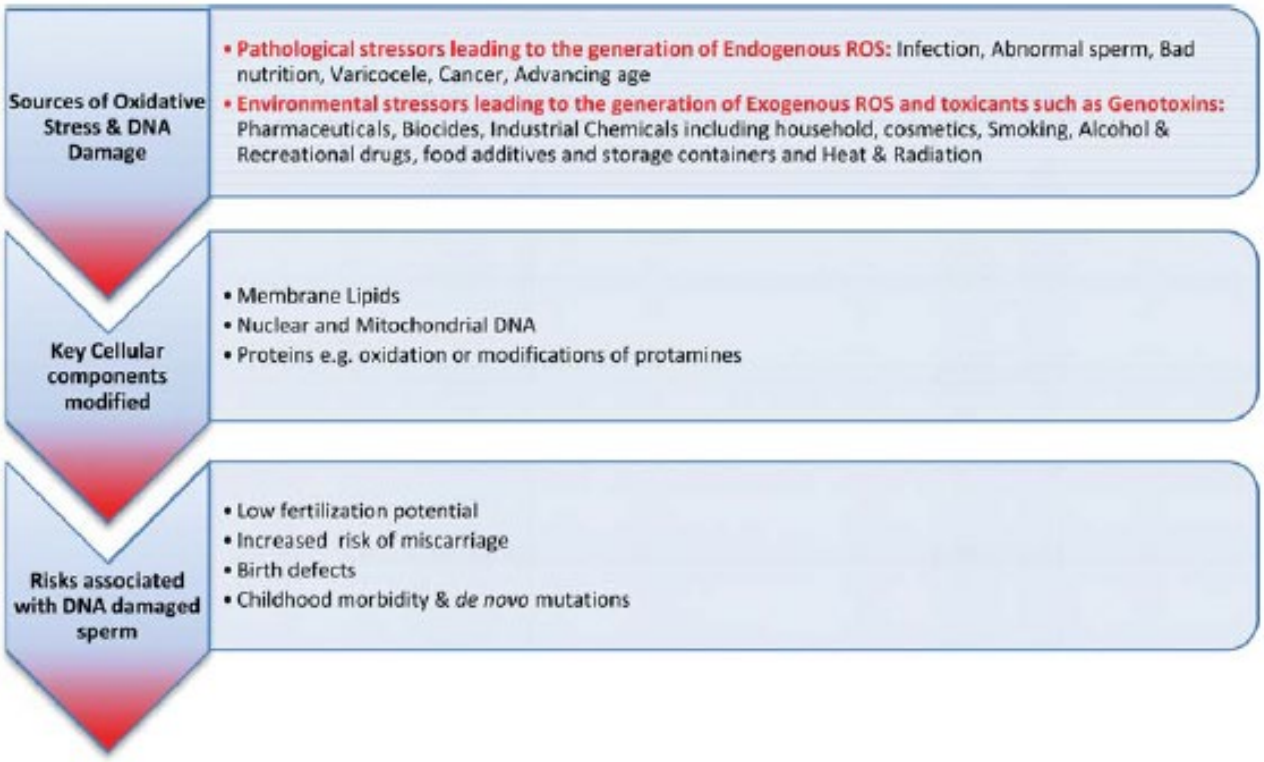


Figure 1 Sperm oxidative stress and DNA damage: its potential consequences for fertility.

Hum Reprod, 2011

Chromatin damages and epigenetic consequences

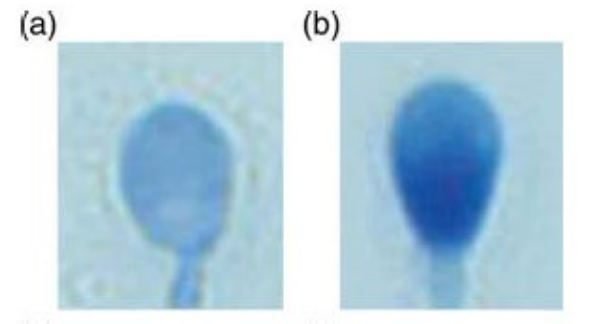
Improvement of gamete quality by stimulating and feeding the endogenous antioxidant system: mechanisms, clinical results, insights on gene-environment interactions and the role of diet

M Dattilo et al, 2016

- Oxidative aggression may affect the chromatin structure and the epigenetic regulation of gametes in at least two relevant manners:
in sperm, by affecting the protamin content; in both sperm cells and oocytes by misleading the epigenetic marks, i.e. by affecting DNA metilation patterns

Aniline blue staining

- The degree of sperm nuclear condensation or maturation can be assessed by aniline blue staining, which discriminates between lysine-rich histones and arginine and cysteine-rich protamines (Stockert & Barrera, 1995).
- According to Dadoune et al. (1988) and Hofmann & Hilscher (1990), a normal ejaculation should contain at least 75% unstained spermatozoa, which indicates a normal nuclear maturation of ejaculated spermatozoa (Dadoune et al., 1988; Hofmann & Hilscher, 1990).



A) A NORMALLY CONDENSED CHROMATIN SPERMATOZOON
B) A NON-CONDENSED CHROMATIN SPERMATOZOON

Boitrelle et al, 2011

Aniline blue staining

- Highlights defects of the **tertiary structure** of chromatine
  - i.e. reduced amounts of protamines, reduced cohesion between protamines

- Expressed as **S**perm **D**econdensation **I**ndex (SDI)
  - N° decondensed sperms (blue colored)/total N° of sperms

Prospective clinical study

Role of enzimatic and non-enzimatic antioxidants on oxidative stress: study on 19 infertile men

Patients

19 male partners of infertile couple with at least 1/1,5 years of natural attemps (no female factor)

	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
Age	33,0	36,0	38,0	38,0	39,5	45,0
GG Astin.	3	3	4	4	5	6
pH	7.2	7.7	8	7.86	8	8.2
Volume	2	2.95	3.6	3.8	4.65	6.5
NumTot	9.9	42.4	89.9	116.49	175.55	341
Conc.	2.1	14.75	27.4	34.22	41.35	170.5
MotAB	15	29.5	38	39.47	48.5	64
pc.Fnorm	0	1	2	2.42	3.5	8
concLeu	0	0	0.1	0.67	0.95	6.3
concGerm	0	0	0.1	0.22	0.3	1.3
HOST.Tot	53	71	89	83.05	94.5	100
HCT	13	37.5	49	49	60.5	89
SDI	11	39.5	51	51	62.5	87

Methods

- **Intervention**

Enzimatic and non-enzimatic antioxidants therapy (once a day) for 1 month

- **Measurements**

Semen analysis according to WHO, 5th edition before therapy (T0) and after 1 month of therapy (T1)

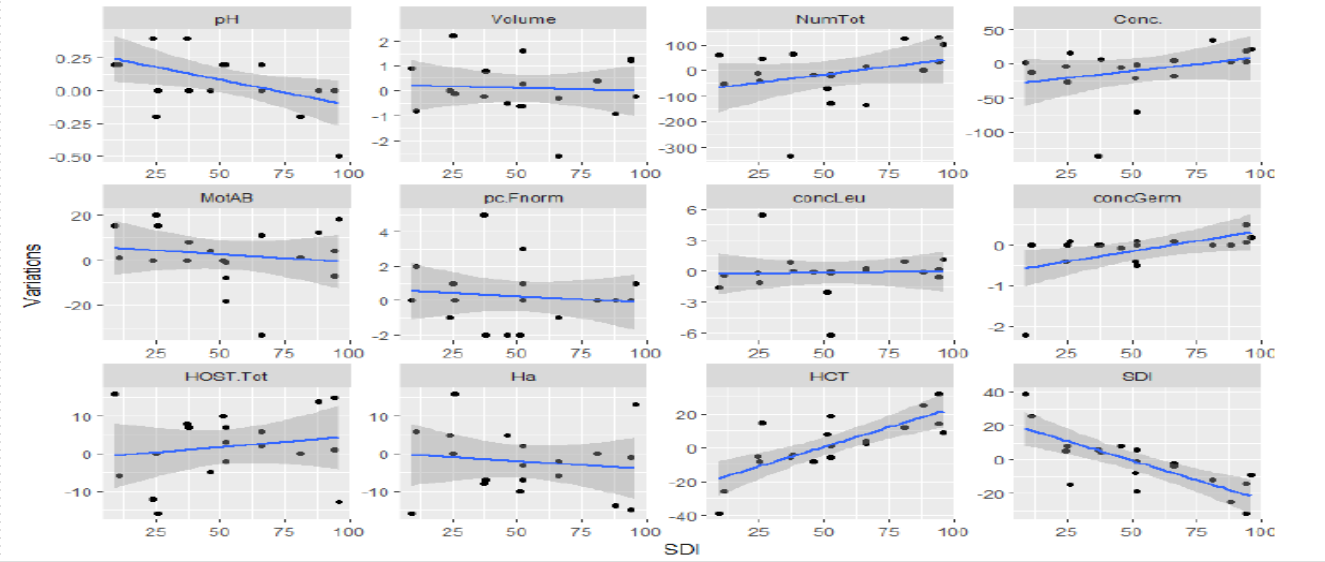
Sperm chromatin Decondensation Index (**SDI**) and Histon Color Test Index (**HCT**) by **aniline blue staining** at T0 and T1

SDI = 1-HCT

HCT = 1-SDI

Vitality Test (**Hypoosmotic Swelling Test** o **HOST**)

Variations at T1 (first follow up) vs SDI at T0



- SDI gives the percentage of immature spermatozoa
- An improving of chromatin maturity is associated to a reduction of SDI (or an encrease of HCT)

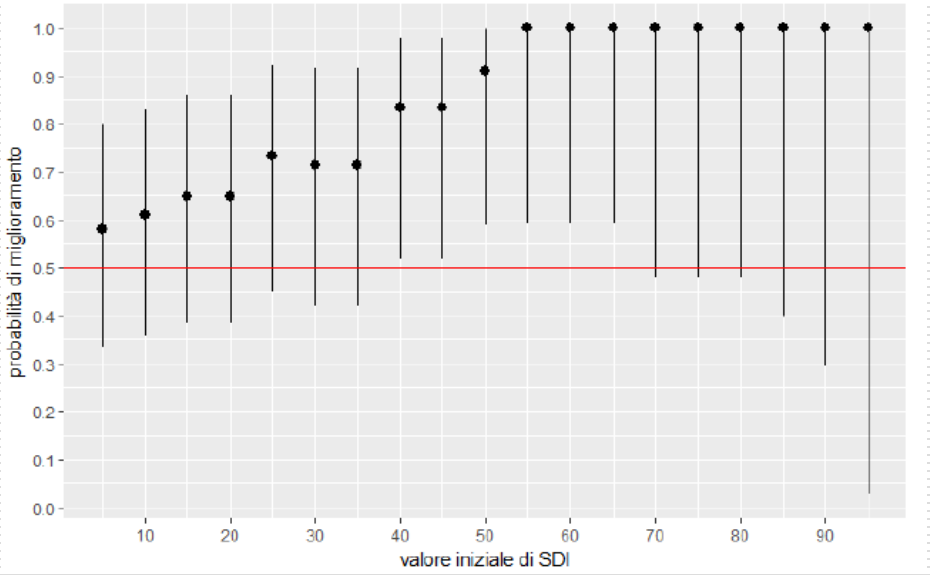
$\alpha = 0{,}05$

Correlations

par	Correlazione con SDI iniziale	P. value - non corretti post hoc-	P value correz post Hoc
HCT	0.76	1e-04	0.0015
concGerm	0.51	0.0255	0.2551
NumTot	0.32	0.1768	1
Conc.	0.30	0.2068	1
HOST.Tot	0.16	0.4905	1
concLeu	0.03	0.8897	1
Volume	-0.06	0.7987	1
pc.Fnorm	-0.11	0.6372	1
Ha	-0.13	0.5914	1
MotAB	-0.15	0.5380	1
PH	-0.50	0.0272	0.2551
SDI	-0.76	1e-04	0.0015

- For statistical evaluation, we considered 12 variables, and 2 of them had a normal distribution (Shapiro-Wilk test, unadjasted p.val >0.98)
- For a better omogeinity, Wilcoxon Test was used
- The correlation is significative after the application of the correction post hoc (Holm Test)

Valutation of cut-off for improving of SDI (CI 95%)



- For SDI values 40 %< SDI < 65% we observed a significant difference (p-value<0,01; CI 95%) between the value of SDI before and after therapy
- For each patient, for SDI > 40% the probability of improvement (that is the decrease of SDI) of the sperm chromatin condensation is more than 83% and statistically significant (p-value < 0,01 binomial test).

Study conclusions

- Sperm Decondensation Index SDI significantly decreases in infertile males when 40<SDI<65% after 1 month therapy with antioxidants

- We strongly recommended the antioxidants treatment to obtain the improving of the sperm chromatin condensation

Study conclusions

- With this study, we found a new range with the aniline blue staining for the diagnosis and successful treatment of the infertile males

- However, the conventional methods are not efficient to evaluate the cases with unexplained infertility. Therefore, in the case of infertility or unexplained infertility, the spermatozoa should be evaluated by new tests in addition to conventional semen analysis, such as the sperm DNA integrity assessment tests and chromatin condensation tests, which examine the protein structure of the chromatin.